Spring 5-9-2016

Genetic engineering of CHO cells for viral resistance to MMV: Targeting virus binding, internalization, intracellular trafficking and transport to nucleus

Joaquina Mascarenhas
SAFC

Genetic Engineering of CHO cells for Viral Resistance to MMV: Targeting Virus Binding, Internalization, Intracellular Trafficking and Transport to Nucleus

Joaquina X Mascarenhas [1], Nikolay Korokhov[2], Ademola Kassim [1], Jason Tuter [1], David Razafsky [1]Trissa Borgschulte [1], Henry George [1], Audrey Chang [2], David Onions [2], Kevin. Kayser [1]

[1]Cell Sciences and Development, SAFC Sigma Aldrich, Saint Louis, Missouri, 63117, USA
[2]Bioreliance, SAFC Sigma Aldrich, Rockville, Maryland, 20850, USA

Contamination by the parvovirus Mouse Minute Virus (MMV) remains a challenge in CHO biopharmaceutical production processes. As part of developing a risk mitigation strategy against such events our group has evaluated the genetic engineering of Chinese Hamster Ovary (CHO) cell lines to create a new host cell line that would be resistant to MMV. While the functional receptor for MMV binding to CHO cell surface is unknown, previous work in our group has validated the role of sialic acid on the cell surface as important for cell surface binding and internalization of the MMV virus. In this work we further characterize the cell surface receptors used by MMV for binding and internalization, as well as the role of various cytoskeletal elements and proteins involved in virus transport and entry into the nucleus.

Sialyltransferases are a group of enzymes that catalyze the transfer of sialic acid to the glycan moiety of glyoconjugates. *In-vitro* binding studies using glycan-arrays have indicated that MMV binds preferentially to α-2,3 linked sialylated glycans and do not bind to α-2,6 sialylated glycans. CHO cells have six different sialyl transferases (ST3Gal 1-6), that transfer sialic acid in a α-2,3 linkage specific manner. In this work we systematically created targeted mutations within genes encoding for the ST3Gal 1,3 4 & 6 sialyltransferases, and then probed for their role in MMV infectivity by challenging each cell line for their ability to resist viral entry. Results indicated that of the sialyltransferases mutations within St3Gal4 had a predominant effect on MMV binding and internalization resulting in a 54-88% decrease in infection compared to the control WT cells. Based on previous work with mutants that created truncated O-glycans, we hypothesized that St3Gal1 knock-outs would have a significant effect on MMV infectivity. We also sought to replace the α-2,3 sialylated phenotype with α-2,6 sialylation on the glycoproteins expressed in the viral resistant host cell lines by over-expression of the St6Gal1 gene. Such an approach would also make the therapeutic protein have more “human” like glycosylation and replace the terminal sialic acid on therapeutic molecules. Once MMV is able to enter the cell, it uses various cellular mechanisms for virus transport to and into the nucleus. We targeted two genes Vimentin and Caspase-3 involved in vesicular transport and virus entry into the nucleus, with the aim of further inhibiting virus replication in CHO host cell lines.

Model recombinant proteins were transfected into the new host cell lines and growth, IgG productivity and critical product quality attributes examined. Our data demonstrate that viral resistance against MMV virus can be incorporated into CHO production cell lines, adding another level of “defense”, against the devastating financial consequences of this virus infection, without compromising recombinant protein yield or quality.